OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Terrestrial Plant Test: Vegetative Vigour Test

INTRODUCTION

1. OECD Guidelines for the Testing of Chemicals are periodically reviewed in the light of scientific progress and applicability to regulatory use. This Guideline, which emerged from the review of the previous Guideline 208 (1), is designed to assess effects on vegetative vigour of terrestrial plants following above-ground exposure. As such it does not cover all chronic effects or effects on reproduction (i.e. seed set, flower formation, fruit maturation). Conditions of exposure and properties of the substance to be tested must be considered to ensure that appropriate test methods are used. This guideline is applicable to the testing of both general chemicals, biocides and crop protection products (also known as plant protection products or pesticides). It has been developed on the basis of Guideline 208 (1)(2) and other existing methods (3) (4) (5) (6) (7) (8). Other references pertinent to plant testing were also considered (9) (10) (11) (12). Definitions used are given in Annex 1.

PRINCIPLE OF THE TEST

2. The test assesses the potential effects on plants following deposition of the test substance on the leaves and above-ground portions of plants. Plants are grown from seed usually to the 2- to 4- true leaf stage. Test substance is then sprayed on the plant and leaf surfaces at appropriate rate(s). After the application, the plants are evaluated against untreated control plants for effects on vigour and growth at various time intervals through 21 - 28 days from treatment. A test period of 21 days can be sufficient for the 10 crop species listed in Annex 4. Endpoints measured are dry shoot weight (alternatively fresh shoot weight), and in certain cases shoot height, as well as an assessment of visible detrimental effects on different parts of the plants. These measurements and observations are compared to those of untreated control plants.

3. The test can be conducted in order to determine the dose-response curve, or at a single concentration/rate as a limit test according to the aim of the study. If results from the single concentration/rate test exceed a certain toxicity level (e.g. whether effects greater than x % are observed), a range finding test is carried out to determine upper and lower limits for toxicity followed by a multiple concentration/rate test to generate a dose-response curve. Appropriate statistical analysis are used to obtain an effective concentration EC_x or an effective application rate ER_x (e.g. EC_{25}, ER_{25}, EC_{50}, ER_{50}) for the most sensitive parameter(s) of interest. Also, the no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) can be calculated in this test.

INFORMATION ON THE TEST SUBSTANCE

4. The following information is useful for the identification of expected route of exposure to the substance and in designing the test: structural formula, purity, water solubility, solubility in organic solvents, n-octanol/water partition coefficient, soil sorption behaviour, vapour pressure, chemical stability in water and light, and biodegradability

VALIDITY OF THE TEST

5. In order for the test to be considered valid, the following performance criteria must be met:

- the seedling emergence is at least 70 %;

and in the controls:

- the plants do not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations). Plants exhibit only normal variation in growth and morphology for that particular species;
- the mean plant survival is at least 90 % for the duration of the study;
- environmental conditions for a particular species are identical and growing media contain the same amount of soil matrix, support media, or substrate from the same source.

REFERENCE SUBSTANCE

6. A reference substance may be tested at regular intervals, to verify that the performance of the test and the response of the particular test plants and the testing facility have not changed significantly over time. Alternatively, historical biomass or growth measurement of controls could be used to evaluate the performance of the test system in particular laboratories, and can serve as an intra-laboratory quality control measure.

DESCRIPTION OF THE METHOD

Natural soil - Artificial substrate

7. Plants may be grown in pots using a sandy loam, loamy sand, or sandy clay loam that contain up to 1.5 percent organic carbon (approx. 3 percent organic matter). Commercial plotting soil or synthetic soil mix that contains up to 1.5 percent organic carbon may also be used. Clay soils should not be used if the test substance is known to have a high affinity for clays. Field soil should be sieved to 2 mm particle size in order to homogenize it and remove coarse particles. The type and texture, % organic carbon, pH and salt content as electronic conductivity of the final prepared soil should be reported. The soil should be classified according to a standard classification scheme (13). The soil could be pasteurized or heat treated in order to reduce the effect of soil pathogens.

8. Natural soil may complicate interpretation of results and increase variability due to varying physical/chemical properties and microbial populations. These variables in turn alter moisture-holding capacity, chemical-binding capacity, aeration, and nutrient and trace element content. In addition to the variations in these physical factors, there will also be variation in chemical properties such as pH and redox potential, which may affect the bioavailability of the test substance (14) (15) (16). Thus, the use of artificial substrates is an acceptable alternative.

9. Artificial substrates are typically not used for testing of crop protection products, but they may be of use for the testing of general chemicals or where it is desired to minimize the variability of the natural soils and increase the comparability of the test results. Substrates used should be composed of inert materials that minimize interaction with the test substance, the solvent carrier, or both. Acid washed quartz sand, mineral wool and glass beads (e.g., 0.35 to 0.85 mm in diameter) have been found to be suitable inert

materials that minimally absorb the test substance (17), ensuring that the substance will be maximally available to the seedling via root uptake. Unsuitable substrates would include vermiculite, perlite or other highly absorptive materials. Nutrients for plant growth should be provided to ensure that plants are not stressed through nutrient deficiencies, and where possible this should be assessed via chemical analysis or by visual assessment of control plants.

Criteria for selection of test species

10. The species selected should be reasonably broad, e.g., considering their taxonomic diversity in the plant kingdom, their distribution, abundance, species specific life-cycle characteristics and region of natural occurrence to develop a range of responses (11) (18) (19) (20) (21) (22). The following characteristics of the possible test species should be considered in the selection.

- the species have uniform seeds that are readily available from reliable standard seed source(s) and that produce consistent, reliable and even germination, as well as uniform seedling growth;
- plant is amenable to testing in the laboratory, and can give reliable and reproducible results within and across testing facilities;
- the sensitivity of the species tested should be consistent with the responses of plants found in the environment exposed to the substance;
- they have been used to some extent in previous toxicity tests and their use in, for example, herbicide bioassays, heavy metal screening, salinity or mineral stress tests or allelopathy studies indicates sensitivity to a wide variety of stressors);
- they are compatible with the growth conditions of the test method;
- they meet the validity criteria of the test.

Some of the historically most used test species are listed in Annex 2 and potential non-crop species in Annex 3.

11. The number of species to be tested is dependent on relevant regulatory requirements, therefore it is not specified in this Guideline.

Application of the test substance

12. The substance should be applied in an appropriate carrier (e.g. water, acetone, ethanol, polyethylene glycol and gum Arabic). Formulated products and formulations containing active ingredients and various adjuvants can be tested as well.

13. All equipment used in conducting the test, including equipment used to prepare and administer the test substance should be of such design and capacity that tests involving this equipment can be conducted in an accurate way and it will give a reproducible coverage. The coverage should be uniform across the leaf surfaces and "shading" of leaves from the substance should not occur. The test substance is sprayed onto the plant surface simulating typical spray tank applications. Generally, spray volumes should be in the range of normal agricultural practice, and not to exceed plant runoff. If solvents or carriers are applied, a second group of control plants should be established receiving only the solvent/carrier. This is not necessary for crop protection products tested as formulations. In the case of test substance under the form of dusts, the test would require further modification.

Verification of test substance concentration/rate

14. The concentrations/rates of application must be confirmed by appropriate analytical verification. For soluble materials, verification of all test concentrations/rates can be confirmed by analysis of the highest concentration test solution used for the test with documentation on subsequent dilution and use of calibrated application equipment (e.g., calibrated analytical glassware, calibration of sprayer application equipment). For insoluble materials verification of compound material must be provided with weights of test material added to the soil. If demonstration of homogeneity is required, analysis of the soil may be necessary.

PERFORMANCE OF THE TEST

Test design

15. Plants of the same species are grown in pots from seeds to the 2- to 4 true leaf stage. The indication of this stage depends on the selected plant species and might not be appropriate for certain species (e.g. onions). However, the exact description of stage used for the test should be documented in the test report. The number of plants per pot will depend upon the species, pot size and test duration, and should provide adequate and uniform growth conditions and avoid overcrowding and shading of plants by each other for the duration of the test. As an example, one to two corn, soybean, tomato, cucumber, or sugar beet plants per 15 cm container; three rape or pea plants per 15 cm container; and 5 to 10 onion, wheat, or other small seeds per 15 cm container are recommended. The number of seeds and replicate pots (the replicate is defined as a pot, therefore plants within the same pot do not constitute a replicate) should be adequate for optimal statistical analysis (23). In certain cases a tray of multiple pots with one plant per pot can also be considered as a replicate. This can reduce within group variability making it easier to detect differences between groups. It should be noted that variability will be greater for test species using fewer large seeds per pot (replicate), when compared to test species where it is possible to use greater numbers of small seeds per pot. By planting equal seed numbers in each pot this variability may be minimized.

16. After the seeds have emerged, thinning should be completed so that there is only one plant per pot for larger-growing species, while for smaller growing species more than one plant per pot is allowed. Whether or not there will be one plant per pot or more than one plant per pot will be dependent on the size the plant will grow to by the end of the test period, and to avoid overcrowding. As much as is possible, there should be only one plant per pot. Thus, depending on the ultimate size the plant, a replicate can be one plant per pot, several plants per pot, or a tray of pots, each with one plant.

17. Control groups are used to assure that effects observed are associated with or attributed only to the test substance exposure. The appropriate control group should be identical in every respect to the test group except for exposure to the test substance. Within a given test, all test plants including the controls should be from the same source. To prevent bias, random assignment of test and control pots is required.

18. Seeds coated with an insecticide or fungicide (i.e. dressed seeds) should be avoided. However, the use of certain non-systemic contact fungicides (e.g. captan, thiram) is permitted by some regulatory authorities (24). If seed-borne pathogens are a concern, the seeds may be soaked briefly in a weak 5 % hypochlorite solution, then rinsed extensively in running water and dried. No remedial treatment with other crop protection products is allowed.

Test conditions

19. The test conditions should approximate those conditions necessary for normal growth or typical environmental conditions for the species and varieties tested (as an example see Annex 4). They should avoid crowding of the plants that could affect growth and overlapping of leaves that could affect exposure to test substance.

20. The plants should be maintained under good horticultural practices in controlled environment chambers, phytotrons or greenhouses. When using growth facilities these practices usually include control and adequately frequent (e.g. daily) recording of temperature, humidity, carbon dioxide concentration, light (intensity, wave length, photosynthetically active radiation) and light period, means of watering, etc., to assure good plant growth as judged by the control plants. Greenhouse temperatures should be controlled through venting, heating and/or cooling systems. The following conditions are generally recommended for greenhouse testing:

- temperature: 22 °C±10 °C;
- humidity: 70 % ±25 %;
- photoperiod: minimum 16h light;
- light intensity: 350±50 $\mu E/m^2/s$. Additional lighting may be necessary if intensity decreases below 200 $\mu E/m^2/s$, wavelength 400-700 nm except for certain species whose light requirements requires less light intensity.

Environmental conditions should be monitored and reported during the course of the study. The plants should be grown in non-porous plastic or glazed pots with a tray or saucer under the pot. The pots may be repositioned periodically to minimize variability in growth of the plants (due to differences in test conditions within the growth facilities). The pots must be large enough to allow normal growth and limit overlap of leaves among plants.

21. Soil nutrients may be supplemented to maintain good plant vigour. The need and timing of additional nutrients can be judged by observation of the control plants. Bottom watering of test containers (e g. by using glass fiber wicks) or watering under the foliage is recommended.

22. The specific growing conditions should be appropriate for the species tested and the test substance under investigation Control and treated plants must be kept under the same environmental conditions, however, adequate means should be taken to prevent cross exposure (e.g. of volatile substances) among different treatments and the controls to the test substance.

Testing at a single concentration/rate

23. In order to determine the appropriate concentration/rate of a substance for conducting a single-concentration or rate (challenge/limit) test, a number of factors must be considered. For general chemicals, these include the physical and chemical properties of the substance. For crop protection products, the physical-chemical properties and use pattern of the test substance, its maximum concentration or application rate, the number of applications per season and/or the persistence of the test compound need to be considered. To determine whether a general chemical possesses phytotoxic properties, it may be appropriate to test at a maximum level of approximated foliar exposure (e g 1000 mg/l test solution).

Range-finding test

24. When necessary a range-finding test could be performed to provide guidance on concentrations or application rates to be tested in definitive dose-response study. For the range-finding test, the test concentrations/rates should be widely spaced (e.g. 0.1, 1.0, 10, 100 and 1000 units of application). For crop protection products concentrations/rates could be based on the recommended or maximum concentration or application rate, e.g. 1/100, 1/10, 1 times of the recommended/maximum concentration or application rate.

Testing at multiple concentrations/rates

25. The purpose of the multiple concentration/rate test is to establish a dose-response relationship and to determine an EC_x or ER_x for biomass and/or visual effects compared to un-exposed controls, as required by regulatory authorities.

26. The number and spacing of the concentrations or rates should be sufficient to generate a reliable dose-response relationship and regression equation and give an estimate of the EC_x or ER_x. The selected concentrations/rates should encompass the EC_x or ER_x values that are to be determined. For example, if an EC_{50} is required it would be desirable to test at rates that produce a 20 to 80 % effect. The recommended number of test concentrations/rates to achieve this is at least five in a geometric series plus untreated control, and spaced by a factor not exceeding 3. For each treatment and control group, the number of replicates should be at least four and the total number of plants should be at least 20. More replicates of certain plants with variable growth habits may be needed to increase the statistical power of the test. If a larger number of test concentrations/rates is used, the number of replicates may be reduced. If the NOEC is to be estimated, more replicates may be needed to obtain the desired statistical power (23).

Observations

27. During the observation period, the plants are observed frequently (at least weekly and if possible daily) for visual phytotoxicity and mortality. At the end of the test, measurement of biomass of surviving plants should be recorded as well as visible detrimental effects on different parts of the plant. The latter include abnormalities in appearance of the young plants, stunted growth, chlorosis, discoloration, mortality, and effects on plant development. The final biomass can be measured using final average dry shoot weight of surviving plants, by harvesting the shoot at the soil surface and drying them to constant weight at 60° C. Alternatively, the final biomass can be measured using fresh shoot weight. The height of the shoot may be another endpoint, if required by regulatory authorities. A uniform scoring for visual injury should be used to evaluate the observable toxic responses. Examples for performing qualitative and quantitative visual ratings are provided in references (12) (25).

DATA AND REPORTING

Statistical analysis

Single concentration/rate test

28. Data for each plant species should be analyzed using an appropriate statistical method (23). The level of effect at the test concentration/rate should be reported, or the lack of reaching a given effect at the test concentration/rate (e.g., <*x* % effect observed at *y* concentration or rate).

Multiple concentration/rate test

29. A dose-response relationship is established in terms of a regression equation. Different models can be used, for example for estimating EC_x or ER_x (e.g. EC_{25}, ER_{25}, EC_{50}, ER_{50}) and its confidence limits for mortality as quantal data, logit, probit, Weibull, Spearman-Karber, trimmed Spearman-Karber methods, etc. could be appropriate. For the growth of the seedlings (weight and height) as continuous endpoints EC_x or ER_x and its confidence limits can be estimated by using appropriate regression analysis (e.g. Bruce-Versteeg non-linear regression analysis (26)). Wherever possible, the R^2 should be 0.7 or higher for the most sensitive species and the test concentrations/rates used encompass 20 % to 80 % effects. If the NOEC is to be estimated application of powerful statistical tests should be preferred and these should be selected on the basis of data distribution (23) (27).

Test report

30. The test report should present results of the studies as well as a detailed description of test conditions, a thorough discussion of results, analysis of the data, and the conclusions drawn from the analysis. A tabular summary and abstract of results should be provided. The report must include the following:

Test substance:

- chemical identification data, relevant properties of the substance tested (e.g. log Kow, water solubility, vapour pressure and information on environmental fate and behaviour if available);
- details on preparation of the test solution and verification of the test concentrations as specified in paragraph 14.

Test species:

- details of the test organism: species/variety, plant families, scientific and common names, source and history of the seed as detailed as available (i.e. name of the supplier, percentage germination, seed size class, batch or lot number, seed year or growing season collected, date of germination rating), viability, etc.;
- number of mono-and di-cotyledon species tested;
- rationale for selecting the species;
- description of seed storage, treatment and maintenance.

Test conditions:

- testing facility e.g. growth chamber, phytotron, greenhouse;
- description of test system (e.g., pot dimensions, pot material, and amounts of soil);
- soil characteristics (texture or type of soil: e.g. soil particle distribution and classification, physical and chemical properties including % organic matter, % organic carbon, pH,);
- soil/substrate (e.g. soil, artificial soil, sand, others) preparation prior to test;
- description of nutrient medium if used;
- application of the test substance: description of method of application, description of equipment, exposure rates and volumes including chemical verification, description of calibration method, description of environmental conditions during application;
- growth conditions: light intensity (e.g. PAR photosynthetically active radiation), photoperiod, max/min temperatures, watering schedule and method, fertilization;

- number of seeds per pot; number of plants per dose; number of replicates (pots) per exposure rate;
- type and number of controls (negative and/or positive controls, solvent control if used);
- stage of the plant development at the start of the test;
- duration of the test.

Results:

- table of all endpoints for each replicate, test rate/concentration and species,
- the percent inhibition for each species as compared to controls;
- biomass measurements, (shoot dry weight or fresh weight) of the plants as a percentage of the controls;
- shoot height of the plants as percentage of the controls, if measured;
- reproductive structure measurements, if present;
- percent visual injury and qualitative and quantitative description of visual injury (chlorosis, necrosis, wilting, leaf and stem deformation, as well as, any lack of effects) by the test substance as compared to control plants;
- a description of the rating scale used to judge visual injury, if visual rating is provided;
- for single rate studies, the % injury should be reported;
- ECx or ERx (e.g. EC_{50}, ER_{50}, EC_{25}, ER_{25}) values and related confidence limits. Where regression analysis is performed, provide the standard error for the regression equation, and the standard error for individual parameter estimate (e.g. slope, intercept);
- NOEC (and LOEC) values if calculated;
- description of the statistical procedures and assumptions used;
- graphical display of these data and dose-response relationship of the species tested

Deviations from the procedures described in this guideline and any unusual occurrences during the test.

LITERATURE

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- 850.4000: Background - Non-target Plant Testing;
- 850.4025: Target Area Phytotoxicity;
- 850.4100: Terrestrial Plant Toxicity, Tier I (Seedling Emergence);
- 850.4200: Seed Germination/Root Elongation Toxicity Test;
- 850.4225: Seedling Emergence, Tier II;
- 850.4230: Early Seedling Growth Toxicity Test.

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ANNEX 1

DEFINITIONS

Crop Protection Products (CPPs) or plant protection product (PPPs) or pesticides is a material with a specific biological activity used intentionally to protect crops from pests (e.g., fungal diseases, insects, competitive plants).

EC_x, x % Effect Concentration or ER_x, x % Effect Rate: The concentration or the rate that results in an undesirable change or alteration of x % in the test endpoint being measured relative to the control (e.g., 25 % or 50 % reduction in shoot weight, final number of plants present, or increase in visual injury would constitute an EC_{25}/ER_{25} or EC_{50}/ER_{50} respectively).

Emergence is the appearance of the coleoptile or cotyledon above the soil surface.

Formulation is the commercial formulated product containing the active substance (active ingredient), also known as final preparation or typical end-use product (TEP).

LOEC (Lowest Observed Effect Concentration) is the lowest concentration of the test substance at which effect was observed. In this test, the concentration corresponding to the LOEC, has a statistically significant effect ($p<0.05$) within a given exposure period when compared to the control, and is higher than the NOEC value.

Non-target plants: Those plants that are outside the target plant area, for crop protection products, this usually refers to plants outside the treatment area.

NOEC (No Observed Effect Concentration) is the highest concentration of the test substance at which no effect was observed. In this test, the concentration corresponding to the NOEC, has no statistically significant effect ($p < 0.05$) within a given exposure period when compared with the control.

Phytotoxicity: Detrimental deviations (by measured and visual assessments) from the normal pattern of appearance and growth of plants in response to a given substance.

Replicate is the experimental unit which represents the control group and/or treatment group. In these studies, the pot is defined as the replicate.

Visual assessment: Rating of visual damage based on observations of plant stand, vigour, malformation, chlorosis, necrosis, and overall appearance compared with a control.

ANNEX 2

LIST OF SPECIES HISTORICALLY USED IN PLANT TESTING

Family	Species	Common names
DICOTYLEDONAE		
Apiaceae (Umbelliferae)	*Daucus carota*	Carrot
Asteraceae (Compositae)	*Helianthus annuus*	Sunflower
Asteraceae (Compositae)	*Lactuca sativa*	Lettuce
Brassicaceae (Cruciferae)	*Sinapis alba*	White Mustard
Brassicaceae (Cruciferae)	*Brassica campestris var. chinensis*	Chinese cabbage
Brassicaceae (Cruciferae)	*Brassica napus*	Oilseed rape
Brassicaceae (Cruciferae)	*Brassica oleracea var. capitata*	Cabbage
Brassicaceae (Cruciferae)	*Brassica rapa*	Turnip
Brassicaceae (Cruciferae)	*Lepidium sativum*	Garden cress
Brassicaceae (Cruciferae)	*Raphanus sativus*	Radish
Chenopodiaceae	*Beta vulgaris*	Sugar beet
Cucurbitaceae	*Cucumis sativa*	Cucumber
Fabaceae (Leguminosae)	*Glycine max (G. soja)*	Soybean
Fabaceae (Leguminosae)	*Phaseolus aureus*	Mung bean
Fabaceae (Leguminosae)	*Phaseolus vulgaris*	Dwarf bean, French bean, Garden Bean
Fabaceae (Leguminosae)	*Pisum sativum*	Pea
Fabaceae (Leguminosae)	*Trigonella foenum-graecum*	Fenugreek
Fabaceae (Leguminosae)	*Lotus corniculatus*	Birdsfoot trefoil
Fabaceae (Leguminosae)	*Trifolium pratense*	Red Clover
Fabaceae (Leguminosae)	*Vicia sativa*	Vetch
Linaceae	*Linum usitatissimum*	Flax
Polygonaceae	*Fagopyrum esculentum*	Buckwheat
Solanaceae	*Solanum lycopersicon*	Tomato
MONOCOTYLEDONAE		
Liliaceae (Amarylladaceae)	*Allium cepa*	Onion
Poaceae (Gramineae)	*Avena sativa*	Oats
Poaceae (Gramineae)	*Hordeum vulgare*	Barley
Poaceae (Gramineae)	*Lolium perenne*	Perennial ryegrass
Poaceae (Gramineae)	*Oryza sativa*	Rice
Poaceae (Gramineae)	*Secale cereale*	Rye
Poaceae (Gramineae)	*Sorghum bicolor*	Grain sorghum, shattercane
Poaceae (Gramineae)	*Triticum aestivum*	Wheat
Poaceae (Gramineae)	*Zea mays*	Corn

ANNEX 3

LIST OF POTENTIAL NON-CROP SPECIES

Annex 3. OECD Potential Species for Plant Toxicity Testing.

NOTE: The following table provides information for 52 non-crop species (references are given in brackets for each entry). Emergence rates provided are from published literature and are for general guidance only. Individual experience may vary depending upon seed source and other factors.

FAMILY Species Botanical Name (English Common Name)	Lifespan[1] & Habitat	Seed Weight (mg)	Photoperiod for germination or growth[2]	Planting Depth (mm)[3]	Time to Germinate (days)[4]	Special Treatments[5]	Toxicity Test[6]	Seed Suppliers[7]	Other References[8]
APIACEAE									
Torilis japonica (Japanese Hedge-parsley)	A, B disturbed areas, hedgerows, pastures (16, 19)	1.7 - 1.9 (14, 19)	L = D (14)	0 (1, 19)	5 (50%) (19)	cold stratification (7, 14, 18, 19) maturation may be necessary (19) germination inhibited by darkness (1, 19) no special treatments (5)	POST (5)		
ASTERACEAE									
Bellis perennis (English Daisy)	P grassland, arable fields, turf (16, 19)	0.09 - 0.17 (4, 19)	L = D (14)	0 (4)	3 (50%) (19) 11 (100%) (18)	germination not affected by irradiance (18, 19) no special treatments (4, 14)	POST (4)	A, D, F	7
Centaurea cyanus (Cornflower)	A fields, roadsides, open habitats (16)	4.1 - 4.9 (4, 14)	L = D (14)	0 - 3 (2, 4, 14)	14 - 21 (100%) (14)	no special treatments (2, 4)	POST (2, 4)	A, D, E, F	7
Centaurea nigra (Black Knapweed)	P fields, roadsides, open habitats (16, 19)	2.4 - 2.6 (14, 19)	L = D (14)	0 (19)	3 (50%) (19) 4 (97%) (18)	maturation may be necessary (18, 19) germination inhibited by darkness (19) no special treatments (5, 14, 26)	POST (5, 22, 26)	A	
Inula helenium Elecampane	P moist, disturbed sites (16)	1 - 1.3 (4, 14, 29)		0 (4, 29)		no special treatments (4)	POST (4)	A, F	
Leontodon hispidus (Big Hawkbit)	P fields, roadsides, disturbed areas (16, 19)	0.85 - 1.2 (14, 19)	L = D (14)	0 (19)	4 (50%) (19) 7 (80%) (18)	germination inhibited by darkness (17, 18, 19) no special treatments (5, 23)	POST (5, 22, 23)		
Rudbeckia hirta (Black-eyed Susan)	B, P disturbed (16)	0.3 (4, 14)	L = D (14)	0 (4, 33)	< 10 (100%) (33)	no special treatments (4, 14, 33)	POST (4, 33)	C, D, E, F	
Solidago canadensis Canada Goldenrod	P pasture, open areas (16)	0.06 - 0.08 (4, 14)	L = D (11)	0 (4)	14 - 21 (11)	mix with equal part sand and soak in 500ppm GA for 24 hrs (11) no special treatments (4)	POST (4)	E, F	
Xanthium pensylvanicum (Common Cocklebur)	A fields, open habitats (16)	25 - 61 (14, 29)		0 (1) 5 (29)		germination may be inhibited by darkness (1) soak in warm water for 12 hrs (29)	PRE & POST (31)	A	
Xanthium spinosum (Spiny Cocklebur)	A open habitats (16)	200 (14)	L = D (14) L > D (6)	10 (6)		scarification (14) no special treatments (6)	PRE & POST (6)	A	
Xanthium strumarium (Italian Cocklebur)	A fields, open habitats (16)	67.4 (14)	L = D (14)	10 - 20 (6, 21)		no special treatments (6, 14, 21)	PRE & POST (6, 21, 28, 31)	A	
BRASSICACEAE									
Cardamine pratensis (Cuckoo Flower)	P fields, roadsides, turf (16, 19)	0.6 (14, 19)	L = D (14)	0 (19)	5 (50%) (19) 15 (98%) (18)	germination inhibited by darkness (18, 19) no special treatments (5, 14, 22)	POST (5, 22)	F	
CARYOPHYLLACEAE									
Lychnis flos-cuculi (Ragged Robin)	P (16)	0.21 (14)	L = D (14)		< 14 (100%) (14, 25)	maturation may be necessary (18) no special treatments (5, 14, 15, 22-26)	POST (5, 15, 22-26)	F	
CHENOPODIACEAE									
Chenopodium album (Lamb's Quarters)	A field margins, disturbed areas (16, 19)	0.7 - 1.5 (14, 19, 34)	L = D (14)	0 (1, 19)	2 (50%) (19)	treatment differs depending on seed colour (19) dry storage dormancy (19) germination inhibited by darkness (1, 18, 19) cold stratification (18) no special treatments (14, 34)	PRE & POST (28, 31, 34)	A	32

CLUSIACEAE *Hypericum perforatum* (Common St John's Wort)	P fields, arable land, open habitats (16, 19)	0.1 - 0.23 (14, 19)	L = D (14)	0 (1, 19)	3 (19) 11 (90%) (18)	germination inhibited by darkness (1, 18, 19) no special treatments (5, 14, 15, 25, 27)	POST (5, 15, 25, 27)	A, E, F	
CONVOLVULACEAE *Ipomoea hederacea* (Purple Morning Glory)	A roadsides, open habitats, corn fields (16)	28.2 (14)	L > D (6, 10)	10 - 20 (6, 10, 21)	4 (100%) (10)	germination not affected by irradiance (1) no special treatments (6, 21)	PRE & POST (6, 12, 21, 28)	A	
CYPERACEAE *Cyperus rotundus* (Purple Nutsedge)	P arable land, pastures, roadsides (16, 30)	0.2 (14)	L = D (14)	0 (1) 10-20 (6, 10)	12 (91%) (10)	germination inhibited by darkness (1) no special treatments (6, 10, 14)	PRE & POST (6, 28, 31)	B	7
FABACEAE *Lotus corniculatus* (Bird's-foot Trefoil)	P grassy areas, roadsides, open habitats (16, 19)	1 - 1.67 (14, 19)	L = D (14)		1 (50%) (19)	scarification (14, 19) germination not affected by irradiance (18, 19) no special treatments (23, 25)	POST (5, 23, 25)	A, D, E, F	
Senna obtusifolia (*Cassia*, Sicklepod)	A moist woods (16)	23 - 28 (9)	L = D (14) L > D (9)	10 - 20 (6, 9)		soak seeds in water for 24 hours (9) scarification (14) seed viability differs depending on colour (1) no special treatments (6)	POST (6, 9)	A	
Sesbania exaltata (Hemp)	A alluvial soil (16)	11 - 13 (9, 14)	L > D (9)	10 - 20 (9, 21)		soak seeds in water for 24 hours (9) germination not affected by irradiance (1) no special treatments (21)	PRE & POST (9, 21, 28, 31)	A	
Trifolium pratense (Red Clover)	P fields, roadsides, arable land (16, 19)	1.4 - 1.7 (14, 19)	L = D (14)		1 (50%) (19)	scarification (14, 18) may need maturation (19) germination not affected by irradiance (1, 19) no special treatments (5)	POST (5)	A, E, F	
LAMIACEAE *Leonurus cardiaca* (Motherwort)	P open areas (16)	0.75 - 1.0 (4, 14)	L = D (14)	0 (4)		no special treatments (4, 14)	POST (4)	F	
Mentha spicata (Spearmint)	P moist areas (16)	2.21 (4)		0 (4)		no special treatments (4)	POST (4)	F	
Nepeta cataria (Catnip)	P disturbed areas (16)	0.54 (4, 14)	L = D (14)	0 (4)		no special treatments (2, 4, 14)	POST (2, 4)	F	
Prunella vulgaris (Self-heal)	P arable fields, grassy areas, disturbed sites (16, 19)	0.58 - 1.2 (4, 14, 19)	L = D (14)	0 (4, 19)	5 (50%) (19) 7 (91%) (18)	germination inhibited by darkness (18, 19) greater germination with larger seeds (1) no special treatments (4, 14, 22)	POST (4, 22)	A, F	
Stachys officinalis (Hedge-nettle)	P grasslands, field margins (19)	14 - 18 (14, 19)	L = D (14)		7 (50%) (19)	no special treatments (5, 14, 22)	POST (5, 22)	F	
MALVACEAE *Abutilon theophrasti* (Velvetleaf)	A fields, open habitats (16)	8.8 (14)	L = D (14)	10 - 20 (6, 10, 21)	4 (84%) (10)	scarification (14) no special treatments (5, 10, 21)	PRE & POST (6, 22, 28, 31)	A, F	
Sida spinosa (Prickly Sida)	A fields, roadsides (16)	3.8 (14)	L = D (14)	10 - 20 (6, 21)		scarification (14) germination not affected by irradiance (1) no special treatments (6, 21)	PRE & POST (6, 21, 28, 31)	A, F	
PAPAVERACEAE *Papaver rhoeas* (Poppy)	A fields, arable land, disturbed sites (16, 19)	0.1 - 0.3 (4, 14, 19, 29)	L = D (14)	0 (4, 29)	4 (50%) (19)	cold stratification & scarification (1, 19, 32) no special treatments (4, 14, 29)	POST (4)	A, D, E, F, G	

POACEAE									
Agrostis tenuis (Common Bentgrass)	lawns, pastures (16)	0.07 (14)	L > D (10)	20 (10)	10 (62%) (10)	germination inhibited by darkness (1, 17-19) no special treatments (10)	POST (10)	A, E	
Alopecurus myosuroides (Foxtail)	A fields, open habitats (16)	0.9 - 1.6 (29, 34)	L = D (14)	2 (29)	< 24 (30%) (34)	scarification (14) treat with 101 mg/L KNO_3 (14) warm stratification (1) germination inhibited by darkness (1) no special treatments (34)	PRE & POST (28, 34)	A	32
Avena fatua (Wild Oats)	A cultivated areas open habitats (16)	7 - 37.5 (14, 30)	L = D (14) L > D (6)	10 - 20 (6, 10)	3 (70%) (18)	scarification (7, 32) darkness inhibits germination (1) cold stratification (1, 18) no special treatments (6, 10, 14)	PRE & POST (6, 10, 28, 31)	A	
Bromus tectorum (Downy Brome)	A fields, roadsides, arable land (16)	0.45 - 2.28 (14, 29)	L = D (14)	3 (29)		maturation period (1, 7, 32) germination inhibited by light (1) no special treatments (14)	PRE & POST (28, 31)	A	
Cynosurus cristatus (Dog's-tail Grass)	P fields, roadsides, open habitats (16, 19)	0.5 - 0.7 (14, 19, 29)	L = D (14)	0 (29)	3 (50%) (19)	germination not affected by irradiance (19) no special treatments (14, 29)	POST (5)	A	
Digitaria sanguinalis (Crabgrass)	A fields, turf open habitats (16)	0.52 - 0.6 (14, 30)	L = D (14)	10 - 20 (21)	7 (75%) 14 (94%) (7)	scarification, cold stratification, & maturation (1, 7, 14, 32) treat with 101 mg/L KNO_3 (14) germination inhibited by darkness (1) no special treatments (21)	PRE & POST (18, 25, 31)	A	
Echinochloa crusgalli (Barnyard Grass)	A (16)	1.5 (14)	L = D (14) L > D (3)	10 - 20 (7, 21)		scarification (7, 32) germination not affected by irradiance (1) no special treatments (3, 14, 21)	PRE & POST (3, 21, 28, 31)	A	
Elymus canadensis (Canada Wild Rye)	P riparian, disturbed sites (16)	4 - 5 (14, 30)	L = D (11)	1 (11)	14 - 28 (11)	no special treatments (2, 11)	POST (2)	C, D, E	
Festuca pratensis (Fescue)	P fields, moist areas (16, 19)	1.53 - 2.2 (16, 19)	L = D (14) L > D (10)	20 (10)	9 (74%) (10) 2 (50%) (19)	no special treatments (10, 19)	POST (10)	A	7
Hordeum pusillum (Little Barley)	A pastures, roadsides open habitats (16)	3.28 (14)				warm stratification (1) germination not affected by irradiance (1)	PRE (31)		7
Phleum pratense (Timothy)	P pastures, arable fields, disturbed sites (16, 19)	0.45 (14, 19)	L > D (10, 14)	0 - 10 (10, 19)	2 (74%) (10) 8 (50%) (19)	germination inhibited by darkness (19) germination not affected by irradiance (17) no special treatments (10, 14, 17, 19)	POST (10)	A, E	
POLYGONACEAE									
Polygonum convolvulus (Black Bindweed)	A open habitats, roadsides (16)	5 - 8 (4, 14, 29)	L = D (20)	0 - 2 (4, 29)		cold stratification for 4 - 8 weeks (1, 2, 4, 20, 29) germination not affected by irradiance (1)	PRE & POST (1, 2, 20, 28, 31)	A	32
Polygonum lapathifolium (Pale Persicaria)	A moist soil (16)	1.8 - 2.5 (14)	L > D (6)		5 (94%) (18)	germination not affected by irradiance (1) germination inhibited by darkness (18) cold stratification (1) no special treatments (5)	PRE & POST (6)	A, E	
Polygonum pennsylvanicum (Pennsylvania Smartweed)	A fields, open habitats (16)	3.6 - 7 (14, 29)		2 (29)		cold stratification for 4 wks at 0 - 5oC (1, 29) germination inhibited by darkness (1)	PRE (31)	A, E	
Polygonum persicaria (Smartweed)	A disturbed areas, arable land (16, 19)	2.1 - 2.3 (14, 19)	L > D (13)	0 (19)	< 14 (13) 2 (50%) (19)	scarification, cold stratification, GA treatment (14) cold stratification, maturation (17-19) germination inhibited by darkness (19) no special treatments (13)	POST (13)	A	32
Rumex crispus (Curly Dock)	P arable fields, roadsides open areas (16, 19)	1.3 - 1.5 (4, 14, 19)	L = D (14, 33)	0 (4, 19, 33)	3 (50%) (19) 6 (100%) (33)	germination inhibited by darkness (18, 19) maturation may be necessary (18) no special treatments (4, 14, 33)	POST (4, 33)	A, E	32

PRIMULACEAE *Anagallis arvensis* (Scarlett Pimpernel)	A arable fields, open areas, disturbed sites (16, 19)	0.4 - 0.5 (4, 14, 19)	L = D (14)		1 (50%) (19)	cold stratification, GA treatment (1, 14, 18, 19, 32) light required for germination (1) no special treatments (2, 4)	POST (2, 4)	A, F	
RANUNCULACEAE *Ranunculus acris* (Common Buttercup)	P arable fields, roadsides, open areas (16, 19)	1.5 - 2 (14, 19, 29)	L = D (14)	1 (29)	41 - 56 (19, 29)	no special treatments (5, 14, 22, 24 - 26)	POST (5, 22, 24-26)		32
ROSACEAE *Geum urbanum* (Yellow Avens)	P hedgerows, moist areas (16, 19)	0.8 - 1.5 (14, 19)	L = D (14)	0 (19)	5 (50%) (19) 16 (79%) (18)	germination inhibited by darkness (18, 19) warm stratification (1) no special treatments (5, 14, 22, 25, 26)	POST (5, 22, 25, 26)	A	
RUBIACEAE *Galium aparine* (Cleavers)	A arable fields, moist areas disturbed sites (16, 19)	7 - 9 (14, 19)	L = D (14)		5 (50%) (19) 6 (100%) (18)	cold stratification (1, 18, 19) germination not affected by irradiance (18, 19) light inhibits germination (1) no special treatments (6, 14)	PRE & POST (6, 28)	A	32
Galium mollugo (Hedge Bedstraw)	P hedgebanks open areas (8)	7 (29)	L = D (14)	2 (29)		no special treatments (5, 14, 22, 24, 26, 29)	POST (5, 22, 24, 26)	A	
SCROPHULARIACEAE *Digitalis purpurea* (Foxglove)	B, P hedgerows open areas (16, 19)	0.1 - 0.6 (4, 14, 19)	L = D (14)	0 (4, 19)	6 (50%) (19) 8 (99%) (18)	germination inhibited by darkness (1, 17-19) no special treatments (4, 22-26)	POST (4, 22 - 26)	D, G, F	
Veronica persica (Speedwell)	A arable fields, open areas, disturbed sites (16, 19)	0.5 - 0.6 (14, 19)	L = D (14)	0 (19)	3 (19) 5 (96%) (18)	germination inhibited by darkness (18, 19) cold stratification (18) no special treatments (14)	PRE & POST (28)	A	32

[1] A = Annuals, B = Biennials, P = Perennials

[2] References 11, 14 and 33 refer to proportion of light (L) and darkness (D) required to induce seed germination. References 3, 6, 9, 10, 13, 20 refer to growing conditions in greenhouses

[3] 0 mm indicates seeds were sown on the soil surface or that seeds need light to germinate

[4] The numbers provided represent the number of days in which a percent of seeds germinated according to provided reference, e.g., 3 days (50%) germination (reference 19)

[5] Duration of maturation and or stratification not always available. Except for cold treatment requirements, temperature conditions are not specified since in greenhouse testing there is limited temperature control. Most seeds will germinate under normal fluctuation of temperatures found in greenhouses

[6] Indicates species was utilized in either a pre-emergence (PRE) and/or post-emergence (POST) plant toxicity test involving herbicides

[7] Provides example(s) of commercial seed suppliers

[8] Provides two alternative reference(s) that were consulted

Seed Suppliers Cited

Supplier ID	Supplier Information
A	Herbiseed New Farm, Mire Lane, West End, Twyford RG10 0NJ ENGLAND +44 (0) 1189 349 464 www.herbiseed.com
B	Tropilab Inc 8240 Ulmerton Road, Largo, FL 33771-3948 USA (727) 344 - 4050 www.tropilab.com
C	Pterophylla - Native Plants & Seeds #316 Regional Road 60, RR#1, Walsingham, ON N0E 1X0 CANADA (519) 586 - 3985
D	Applewood Seed Co. 5380 Vivian St , Arvada, CO 80002 USA (303) 431 - 7333 www.applewoodseed.com
E	Ernst Conservation Seeds 9006 Mercer Pike, Meadville, PA 16335 USA (800) 873 - 3321 www.ernstseed.com
F	Chiltern Seeds Bortree Stile, Ulverston, Cumbria LA12 7PB ENGLAND +44 1229 581137 www.chilternseeds.co.uk
G	Thompson & Morgan P O. Box 1051, Fort Erie, ON L2A 6C7 CANADA (800) 274 - 7333 www.thompson-morgan.com

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ANNEX 4

EXAMPLE FOR APPROPRIATE GROWTH CONDITIONS FOR CERTAIN CROP SPECIES

The following conditions have been found suitable for 10 crop species, and can be used as a guidance for tests in growth chambers with certain other species as well:

- Carbon dioxide concentration: 350±50 ppm;
- Relative humidity: 70±5 % during light periods and 90±5 % during dark periods;
- Temperature: 25±3^{0}C during the day, 20±3^{0}C during the night;
- Photoperiod: 16h light/8h darkness, assuming an average wavelength of 400 to 700 nm;
- Light: luminance of 350±50 µE/m^2/s, measured at the top of the canopy.

The crop species are:

- tomato (*Solanum lycopersicon*),
- cucumber (*Cucumis sativus*),
- lettuce (*Lactuca sativa*),
- soybean (*Glycine max*),
- cabbage (*Brassica oleracea var. capitata*),
- carrot (*Daucus carota*),
- oats (*Avena sativa*),
- perennial ryegrass (*Lolium perenne*),
- corn (*Zea mays*),
- onion (*Allium cepa*).